Attorney's Docket No.: 56446-20109.00/D1590-2US

.IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Short, et al.
Serial No.: 09/997,807
Filed: November 30, 2001

Art Unit : 1646
Examiner : Michael Borin, Ph.D.

Title: METHOD OF MAKING A PROTEIN POLYMER AND USES OF THE POLYMER

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

1. I, Nelson Barton, am a co-inventor with Jay Short, Eric Mathur, W. Michael Lafferty and Kevin Chow, on the above-identified patent application.

2. I am an expert in the field of polymer chemistry and was an expert at the time of the invention. I am presently employed as a research scientist at Diversa Corporation, San Diego, CA, assignee of the above-referenced patent application. My resume is attached as documentation of my credentials.

3. Examples 19 and 20 of the specification set forth experimentation demonstrating that SEQ ID NO:2 monomers can self-assemble into polymers. These examples, together with the associated disclosure throughout the specification, would have been sufficient to demonstrate how to make and use the presently claimed invention to one of skill in the art. As is indicated in these examples, polymer fibers comprised of the polypeptide of SEQ ID NO:2 were prepared in accordance with particular conditions and reactants.

4. It is my understanding that the Office has questioned whether self polymerization of the presently described monomeric subunits can occur when the subunit includes known modifications, or is attached to an enzyme, a nucleotide, a nucleotide derivative a lipid, a lipid derivative, a targeting molecule and/or a vector (together referred to herein as

conjugated / modified polypeptide monomers). It is my further understanding that the Office has inquired under what conditions will this self assembly of the conjugated / modified polypeptide monomers occur. The state of the art at the time of the invention and the level of skill of the person of ordinary skill in the art for producing polypeptide polymers was very high. Given the disclosure provided in the specification (*see e.g.*, page 101, line 7 to page 102, line 3; Examples 19-21; Figures 2-3), one of skill in the art would also understand how to make and use polymers comprised of conjugated / modified polypeptide monomers. One of skill in the art could determine alternative conditions to achieve self-assembly of the conjugated / modified monomers given the disclosure provided in the specification. It was considered routine by one skilled in the art to determine what conditions to use or modify, including what modifications or attachments are possible or preferred, to produce a protein polymer tubule given the starting materials set forth in the present application. Methods for making and screening for polypeptide polymers comprised of at least one conjugated / modified monomer of SEQ ID NO:2 were sufficiently comprehensive, routine and predictable at the time of the invention to predictably generate a genus of polypeptide polymers without need of knowing which modifications or attachments would detrimentally affect self-polymerization.

5. It is my understanding that the Examiner has interpreted Example 20 as describing merely the polymerization of a crude mixture of proteins from an E. coli extract. In contrast, as would be evident to one of skill in the art, Example 20 contains two precipitation/centrifugation steps that purify the extract before polymerization. *See* pages 149-150 of the specification. The first precipitation is performed utilizing heat treatment, which denatures and precipitates the E. coli proteins. These proteins are then removed by centrifugation, which leaves a soluble CanA fraction that self-assembles. As CanA comes from a hyperthermophile, it can withstand the heat treatment step. In addition, Example 20 describes the performance of additional ammonium sulfate precipitation, followed by another centrifugation. These precipitation and centrifugation steps further purify the supernatant/extract. Accordingly, Example 20 describes an appreciably different process than the polymerization of a crude E. coli extract.

6. As evidence that the methods and materials set forth in the application as filed enable one of skill in the art to practice the invention as presently claimed, I have included (as Exhibit A) an immunofluorescent light microscope image of nanotubules assembled from a polypeptide conjugate of the present invention generated by attaching the CanA open reading frame (SEQ ID NO:1) to the open reading frame of the green fluorescent protein ZSGREEN(TM) (BD Biosciences Clontech, Palo Alto, Calif.). These nanotubules are comprised of monomeric polypeptide subunit conjugates of SEQ ID NO:2 attached to the green fluorescent protein. Self polymerization of the monomeric polypeptide subunit conjugates was achieved under the conditions set forth in Example 20.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted

Date: August 19, 2004

Nelson Barton

sd-214946

NELSON R. BARTON

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	YEARS	FIELD OF STUDY
University of California Berkeley	B.A.	1980-1984	Molecular Biology
University of Miami Sch of Medicine	Ph.D.	1985-1990	Cellular & Molec. Biology
Harvard University	Postdoctoral	1990-1993	Biochemistry & Genetics
University of California San Diego, HHMI	Postdoctoral	1994-1996	Biochemistry & Genetics

Research and Professional Experience

11/1990 – 12/1993 Postdoctoral Fellow, Harvard University, Department of Cell & Developmental Biology. Under direction of Dr. Lawrence Goldstein. Biochemical characterization of microtubule-associated motor proteins involved in chromosome segregation.

1/1994 – 4/1996 Howard Hughes Fellow in the laboratory of Dr. Lawrence Goldstein, Howard Hughes Medical Institute, Div. Of Cellular and Molecular Medicine, Dept. Pharmacology, University of California, San Diego. Biochemical characterization of microtubule-associated motor proteins involved in chromosome segregation.

4/1996 – 05/2000 Manager, Biologicals, R&D, Calbiochem Corporation, San Diego, CA
Developed line of recombinant glycosyltransferases for enzymatic synthesis of oligosaccharides.

5/2000 – 12/2002 Sr. Staff Scientist, Diversa Corporation, San Diego, CA.

1/2003-Present Principal Scientist, Diversa Corporation, San Diego, CA
Currently developing high-throughput screening methods for the discovery and optimization of novel enzymes and biomaterials for use in agriculture, chemical processing, industrial and pharmaceutical applications.

Selected Publications

1. Afshar, K., **N.R. Barton**, R.S. Hawley, and L.S.B. Goldstein (1995). DNA binding and meiotic spindle localization of *Drosophila* NOD kinesin-like protein. Cell 81, 129-138.

2. **Barton, N.R.**, A.J. Pereira, and L.S.B. Goldstein (1995). Motor activity and mitotic spindle localization of the *Drosophila* kinesin-like protein KLP61F. Molec. Biol. Cell 6, 1563-1574

3. **Barton, N.R.** and L.S.B. Goldstein (1996). Going mobile: microtubule motors and chromosome segregation. Proc. Natl. Acad. Sci. (USA) 93, 1735-1742.

4. Ciofalo, V., **N.Barton**, K. Kretz, J. Baird, M. Cook, D. Shanahan (2003). Safety evaluation of a phytase, expressed in *Schizosaccharomyces pombe*, intended for use in animal feed. Reg. Tox. Pharm. 37, 286-292.

5. Garrett, J.B., K.A. Kretz, E. O'Donoghue, J. Kerovuo, W. Kim, **N.R. Barton**, G.P. Hazlewood, J.M. Short, D.E. Robertson, K.A. Gray (2004). Enhancing the thermal tolerance and gastric performance of a microbial phytase for use as a phosphate-mobilizing monogastric feed supplement. Appl. Environ. Microbiol. 70, 3041-3046.